

The Functional Morphology of Starfish Tube Feet: The Role of a Crossed-Fiber Helical Array in Movement

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Abstract. The morphology and mechanics of the tube feet, ampullae, and lateral and radial canals of the water vascular systems of *Luidia clathrata* and *Astropecten articulatus* (Echinodermata, Asteroidea) were analyzed. Histological methods, based on embedding in both paraffin and glycol methacrylate, were used to document the arrangement of muscle and connective tissue. The tube foot wall includes longitudinal muscles and connective tissue fibers, the latter arranged in a crossed-fiber helical array, with a fiber angle of about 67° in elongated tube feet. No evidence was found for the circular rings of connective tissue reported in earlier studies; the appearance of rings is probably an artifact of folding. The ampullae are bilobed and include circumferentially arranged muscle fibers and connective tissue fibers aligned 90° to the muscle. The lateral canals are short and equipped with one-way flap valves similar to those described for other echinoderms. The radial canal is thin-walled, nonmuscular, and enclosed in the connective tissue and ossicles of the ambulacrum. Frame-by-frame video analysis of both intact animals and animals with “windows” cut in the arm wall was used to document the movements of the tube feet and ampullae. No evidence was found for the previously suggested role of the radial canal in protracting the tube feet. The ampullae protract the tube feet and antagonize the tube foot musculature. The fiber angle of the connective tissue allows protraction and prevents dilation of the tube feet, and limits elongation of the ampullae.

Introduction

The water vascular system of asteroids serves crucial roles in locomotion, food handling, respiration and, in

many species, burrowing. The major components of the system are the circumoral ring canal, the radial canals extending from the ring canal down each arm, and the tube feet with their associated ampullae that are connected to the radial canal by the lateral canals. Studies of the tube feet and associated ampullae in asteroids have included analyses of general morphology and function (Mangold, 1908; Hamilton, 1921; Paine, 1926, 1929; Smith, 1937, 1946, 1947; Kerkut, 1953; Heddle, 1967; Nichols, 1966, 1969, 1972), of innervation and neuromuscular control (Smith, 1945, 1950a, b; Bargmann *et al.*, 1962; Cobb, 1967, 1970, 1987; Cobb and Laverack, 1967; Cavey and Wood, 1981), of ultrastructure (Souza Santos and Silva Sasso, 1968, 1970; Dolder, 1972; Engster and Brown, 1972; Wood and Cavey, 1981), and of permeability and maintenance of fluid volume (Binyon, 1962, 1964; Prusch and Whoriskey, 1976; Prusch, 1977; Ferguson, 1990a, b). Although the tube foot–ampulla complex of asteroids has been well studied, two crucial aspects of its function have not been completely resolved. The first concerns connective tissue fiber reinforcement of the walls of the complex, and the second concerns the role of the radial canal in tube foot movements.

The production of force and movement in these appendages depends on a hydraulic mechanism in which contraction of muscle displaces water vascular fluid from one portion of the system to another. In most hydrostatic skeletal support systems that rely on such a mechanism, the walls of the hydraulic appendage or body are reinforced with connective tissue fibers arranged in a specific pattern, referred to as a ‘crossed-fiber helical array’ (for reviews, see Chapman, 1958; Clark, 1967; Trueman, 1975; Wainwright *et al.*, 1976; Alexander, 1983). In such a system, sheets of connective tissue fibers wrap the structure in regular arrays of right- and left-hand helices. The reinforcement provided by the fibers allows shape change

Received 13 May 1994; accepted 10 January 1995.

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and bending and prevents torsion. Although crossed-fiber helical arrays of connective tissue fibers in the walls of the tube feet of ophiuroids have been described and analyzed (Woodley, 1967, 1980), previous studies of the tube feet of asteroids do not describe crossed-fiber helical reinforcement of the tube foot walls. Instead, the connective tissue reinforcement has been reported as consisting of circular rings (Smith, 1946, 1947). The present study was therefore undertaken to reexamine the connective tissue and muscle in the tube feet of two asteroid species, *Luidia clathrata* and *Astropecten articulatus*. Our analysis has revealed crossed-fiber helical connective tissue reinforcement of the tube foot wall, as in other hydraulic systems.

Many of the previous studies cited above have emphasized the interdependence of the tube foot and ampulla in movement; *i.e.*, extension of the tube foot results from contraction of the ampulla, and distension of the ampulla results from contraction of the tube foot. However, recent reviews (Nichols, 1969, 1972) of tube foot functional morphology describe the radial canal in asteroids (species not specified) as being directly involved in extending the tube feet and being capable of accommodating water vascular fluid from contracted tube feet. Indeed, Lawrence (1987, citing Nichols, 1972) describes the radial canal as being responsible for most of the elongation of the tube feet in asteroids. But in the species analyzed in the present study, the radial canal is unlikely to serve in these roles.

Materials and Methods

Experimental animals

Specimens of the grey sea star, *Luidia clathrata*, and the margined sea star, *Astropecten articulatus*, were supplied by Gulf Specimen Supply, Inc., Panacea, Florida. They were maintained in a recirculating artificial seawater system in the Department of Biology, University of North Carolina, Chapel Hill.

Histology

Segments of the arms of *L. clathrata* and *A. articulatus* were removed from specimens that had been anesthetized with a 1:1 mixture of 7.5% $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and seawater (Messenger *et al.*, 1985). The tissue was fixed in 10% formalin in seawater for 24 h. The tissue was then decalcified in a solution of 0.7 g/l ethylenediaminetetraacetic acid, tetrasodium; 8 mg/l sodium potassium tartrate; 99.2 ml/l hydrochloric acid; 0.14 g/l sodium tartrate (S/P Decalcifying Solution, Baxter Scientific Products, McGaw Park, IL) and then washed in water for 2 h. The fixed and decalcified arm tissue was cut into segments that included three to four pairs of tube feet, and these tissue blocks were embedded in paraffin (*L. clathrata* and *A. articulatus*) or glycol methacrylate plastic (*L. clathrata*).

For paraffin embedding, the fixed, decalcified tissues were dehydrated in ethanol, cleared in Histo-Clear (National Diagnostics, Manville, NJ), and embedded in Paraplast Plus (MP 56°C) (Monoject Scientific, St. Louis, MO). The blocks were then serially sectioned on a rotary microtome at 10 μm in three mutually perpendicular planes. The sections were stained with picroponceau with Weigert iron hematoxylin (see Kier, 1992). For glycol methacrylate embedding, the fixed and decalcified tissues were partially dehydrated in an ethanol series to 95% ethanol and then infiltrated with unpolymerized glycol methacrylate plastic (Reichert-Jung Histo-resin, Leica Instruments GmbH, Heidelberg, Germany). Following polymerization, the blocks were sectioned at 0.5–3.0 μm with glass knives. The sections were stained with Lee's methylene blue–basic fuchsin stain (Bennett *et al.*, 1976) and were examined with brightfield, phase contrast, and polarized light microscopy.

In addition to the sectioned material, whole mounts were also examined with brightfield, phase contrast, and polarized light microscopy. Tube feet and ampullae were dissected from fixed and decalcified arm tissue and were partially macerated in 1.0 M potassium hydroxide and 50% glycerine for 2–3 days (Woodley, 1967). The epidermis was removed from the tube feet, and whole mounts of the tube feet and ampullae were prepared.

Computer-assisted three-dimensional reconstruction

The morphology of the valve located between the tube foot–ampulla complex and the radial canal was examined with the aid of a computer program for three-dimensional reconstruction (PC3D, Jandel Scientific, Corte Madera, CA). Parasagittal sections (defined here as vertical planes parallel to the long axis of the arm) and frontal sections (defined here as horizontal planes) were used for the reconstructions of *L. clathrata* tissues. A microscope equipped with a camera lucida was used to trace, from sections, the outline of the internal and external surface of the tube foot, the profile of the valve tissue, and the position of the valve muscle fibers. The tracings were aligned according to the visual best-fit method (Gaunt and Gaunt, 1978; Young *et al.*, 1985) and digitized with a Numonics 2210 digitizing tablet. The PC3D software stacked the tracings of the internal structures to produce a three-dimensional image that could be viewed in any orientation with a Gateway 2000 4DX2-66V microcomputer. The reconstructions were plotted with a Hewlett-Packard HP 7475A plotter.

Video recordings

Locomotion and feeding movements of *L. clathrata* in a glass-bottom aquarium were videotaped from the side and from below with a Panasonic AG-450 S-VHS camera–

recorder. In addition, movements of the tube feet during burrowing were recorded by placing the animals in the aquarium, but with a thin layer of sand on the bottom, and filming from below. The movements were analyzed frame by frame with a Panasonic AG-1960 videocassette recorder.

Direct observations of ampullae

Under anesthesia, the distal portion of an arm and a portion of the dorsal body wall of *L. clathrata* were removed. After such an operation, the animals appear to behave normally. Movements of the ampullae and their associated tube feet were observed directly, both during normal movement of the animal and in response to manual mechanical stimulation of individual tube feet with a dissecting probe.

Results

Morphology of the tube foot-ampulla complex

This study focuses on the components of the water vascular system associated with the radial canal: the tube feet, ampullae, and lateral canals (see Fig. 1). The following morphological description is based on *L. clathrata*. Any observed differences between *Liudia* and *Astropecten* are noted below.

Tube feet. The tube feet project from the ambulacral groove on the ventral surface of the arm; they are cylindrical and have conical ends. Each arm has more than 100 tube feet arranged in pairs along the length of the arm, thus constituting two parallel rows, one on either side of the ambulacral groove. Most of the tube foot is external to the body wall (Fig. 2).

The tube foot epithelium is covered by a thin cuticle continuous with that covering adjacent areas of the ambulacrum (See Engster and Brown, 1972, for details). The appearance of the epithelium in sectioned histological material depends on the state of elongation or contraction of the tube foot. In retracted tube feet, the epithelium is thick and the epithelial surface is highly folded into annular rings (Fig. 3). In protracted tube feet, the epithelium appears thinner and the folding is reduced. The epithelium of the distal conical end appears to be secretory; it consists of tall columnar epithelial cells with intensely staining cell inclusions (Fig. 3).

Underlying the epithelium is a layer of nervous tissue, similar in disposition to the basiepithelial (ectoneural) plexus described previously in other echinoderms (see Smith, 1937; Coleman, 1969; Raymond, 1979). Underlying the nervous tissue layer is a dense layer of fibrous connective tissue. Fibers in this layer show staining reactions typical of collagen and are highly birefringent when viewed with polarized light microscopy. Grazing sections

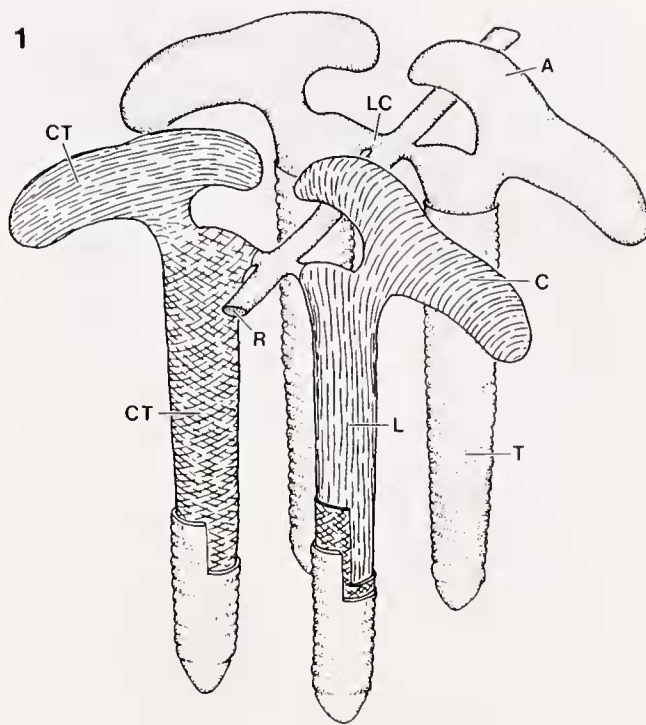


Figure 1. Schematic cutaway drawing of the arrangement of the tube feet (T), ampullae (A), and the lateral (LC) and radial canals (R), showing the trajectories of the connective tissue fibers (CT), longitudinal muscle (L) of the tube feet, and circular muscle (C) of the ampullae.

through the connective tissue layer show that it is composed of connective tissue fibers arranged as a crossed-fiber helical array (Fig. 4). Such an array consists of connective tissue fibers that wrap the tube foot in both left- and right-hand helices. The fiber angle, defined as the angle that the connective tissue fibers make with the long axis of the tube foot, was measured in whole mounts and in grazing sections of protracted tube feet; the angle is about 67° and is relatively constant along the length of the tube foot. In retracted tube feet, the crossed-fiber helical connective tissue layer becomes somewhat folded, and grazing sections may then sometimes give the misleading impression that this layer consists of circumferential rings of connective tissue. Because the fibers are highly birefringent, their arrangement as a crossed-fiber helical array is more easily observed with polarized light microscopy (Fig. 4). The whole-mount preparations were also useful in visualizing the disposition of the connective tissue fibers.

Internal to the crossed-fiber helical connective tissue layer is a robust layer of muscle fibers (see Dolder, 1972, for details) (Figs. 2, 3, 4). The fibers are arranged longitudinally, parallel to the long axis of the tube foot. No striations were observed in the muscle cells. The internal lumen of the tube foot is lined with epithelium (see Wood and Cavey, 1981, for details).

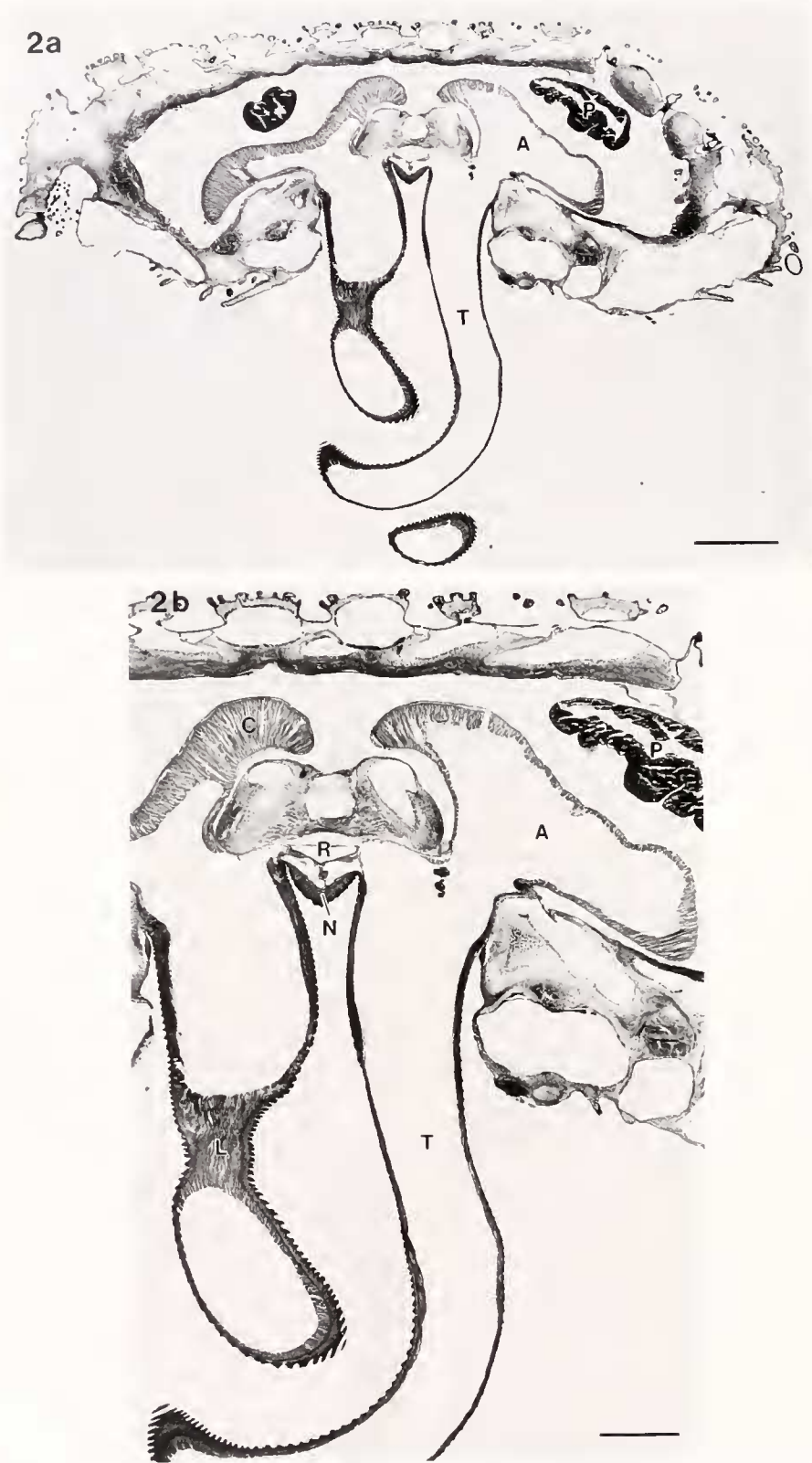


Figure 2. Photomicrographs of a transverse section of an arm from *Luidia clathrata* in the region of a pair of tube feet (T) and their associated bilobed ampullae (A). A 10- μ m-thick paraffin section stained with picro-ponceau and iron hematoxylin was photographed with brightfield illumination. (a) Low power view showing relative proportions of the arm and tube foot-ampulla complex. The section includes a portion of the pyloric caecum (P) on each side of the arm. Scale bar, 1 mm. (b) Higher power view showing the radial canal (R) located dorsal to the radial nerve cord (N). The epithelium on the roof of the radial canal has

Ampullae. Located within the coelomic cavity of the arm are the bulbous ampullae. In both *L. clathrata* and *A. articulatus* the ampullae are bilobed, one lobe extending laterally from the union with the tube foot and the other extending medially (Figs. 2, 5). Each lobe is elongate, cylindrical, and curved toward the oral surface. In *L. clathrata*, the lateral lobe is longer than the medial lobe, whereas in *A. articulatus* the medial lobe is the longer. The long axes of the two lobes are roughly parallel and in the same plane, and so form what is essentially a single cylindrical tube more or less perpendicular to the long axes of both the tube foot and the arm. The connection between the tube feet and the ampullae is made by a slightly narrowed neck that is displaced laterally relative to the axis of the tube foot (Figs. 2, 5). Two bands of tissue, which Smith (1950b) named 'seams' (see also Cobb, 1967; Cobb and Laverack, 1967), run dorsoventrally in the ampullar neck: one seam occupies a medial position on the portion of the neck closest to the central axis of the arm, and the other is located laterally on the opposite side of the ampullar neck (Fig. 5). The tube foot and ampullar wall are continuous through the neck region.

A layer of epithelium covers the outer surface of the ampulla (*i.e.*, the surface exposed to the coelomic cavity of the arm). Beneath the epithelium is the ectoneural nervous tissue layer. Beneath the nervous tissue layer is a thin layer of dense, fibrous, birefringent connective tissue that shows staining reactions typical of collagen. The connective tissue fibers of this layer are closely packed and aligned in parallel. Grazing sections show the fibers to be oriented parallel to the long axis of the ampulla (Fig. 6). Underneath the dense connective tissue sheet is a robust layer of muscle fibers. The muscle fibers are arranged in circumferential bands around the lumen of the ampullae in planes perpendicular to the long axis of the ampulla. Thus, the muscle cells are arranged at right angles to the connective tissue fibers of the dense connective tissue layer. No striations were observed in the muscle cells. The lumen of the ampulla is lined with a simple epithelium.

Lateral canal and valve. Short lateral canals extend from each side of the radial canal to connect to each tube foot along the length of the arm (Fig. 7). The internal lumen of the lateral canals is lined with a simple squamous epithelium that appears to be continuous with that of the tube foot and the radial canal. The canals are wrapped by fibrous birefringent connective tissue continuous with the connective tissue that surrounds the arm ossicles and forms the structure of the arm.

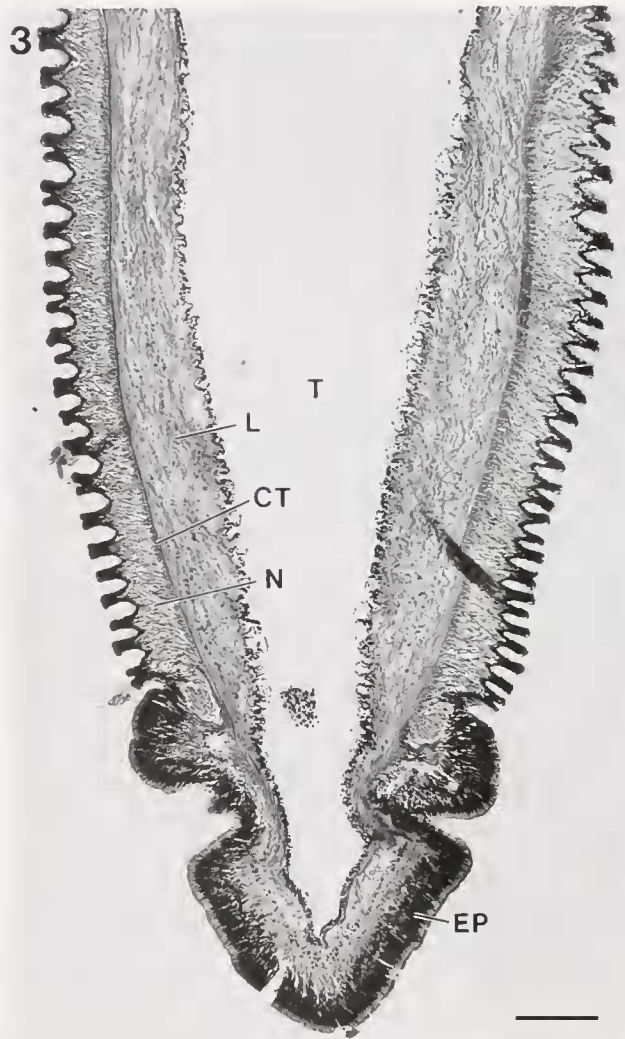


Figure 3. Photomicrograph of a longitudinal section of an individual tube foot (T) from *Luidia clathrata*. The epithelium (EP) of the tip includes tall columnar cells. Folding of the epithelium on the sides of the foot is visible. Underlying the epithelium is a layer of nervous tissue (N), and underneath this is the crossed-fiber helical connective tissue layer (CT). Longitudinal muscle (L) is visible under the connective tissue layer and is separated from the tube foot lumen (T) by a thin epithelium. Scale bar, 100 μm . A 10- μm -thick paraffin section stained with picroponceau and iron hematoxylin was photographed under brightfield illumination.

The lateral canal joins the tube foot wall at the top of the tube foot. A pair of flaps are present on either side of the opening of the lateral canal into the tube foot, forming a valve (Figs. 7, 8). The flaps are parallel to one another

pulled away from the ambulacral connective tissue; glycol methacrylate sections (see Fig. 9) show the epithelium to be attached to the ambulacral connective tissue. The longitudinal muscle (L) of the tube foot (T) and the circumferential muscle (C) of the ampulla (A) are also visible. A portion of a pyloric cecum (P) is visible. Scale bar, 0.5 mm.



Figure 4. Photomicrograph, taken under polarized light, of a partially macerated portion of a tube foot wall from *Luidia clathrata*. The long axis of the tube foot is oriented vertically. The crossed lines indicate the approximate trajectories of the crossed-fiber helical connective tissue array. The longitudinal muscles of the tube foot wall are oriented vertically in the photomicrograph and can be seen behind the crossed fibers. Scale bar, 25 μ m.

and extend down from the roof of the tube foot and laterally from the medial wall of the tube foot on either side of the opening of the lateral canal into the tube foot. Muscle fibers originate on the tube foot roof and wall and insert on the side of the flap opposite to that facing the opening of the lateral canal (Figs. 7, 8). The trajectory of these muscle fibers is such that their contraction pulls the two flaps away from one another, opening the connection between the lateral canal and the tube foot. The flaps consist of a thin sheet of connective tissue covered by a simple squamous epithelium that appears to be continuous with the epithelium of the tube foot and lateral canal. On either side of the valve, the roof of the tube foot is slightly domed.

Radial canal. The radial canal is adjacent to the ventral surface of the arm and extends from the ring canal to the tips of the arms. It is encased in the connective tissue and calcite ossicles of the ambulacrum and is dorsoventrally flattened in cross section (Fig. 9). The radial canal is lined with a simple squamous epithelium, lacks musculature, and is surrounded by connective tissue. The connective tissue is continuous with that surrounding the calcite ossicles of the ambulacrum. No valves or sphincter muscles were observed along its length. The floor of the radial canal is raised as a transverse ridge midway between each pair of tube feet along the length of the arm. The ridges are formed by a bundle of connective tissue and muscle fibers, called 'transverse ambulacral muscles' (Hyman, 1955), that connect ambulacral ossicles on opposite sides of the arm.

Tube feet and ampullae kinematics

Two general categories of movement were observed in the tube feet: length change and bending. As for length change, the tube feet of a large *L. clathrata* (arm length = 9 cm) are about 12 mm long when fully protracted and 2 mm when retracted. During such a retraction, the diameter of the tube foot, measured midway between the base and tip, increases from 0.9 to 1.3 mm. The tube foot wall is opaque, white, and thrown into a series of closely spaced annular folds when retracted. When protracted, the folding is reduced, and the tube foot wall becomes translucent.

The tube feet may bend either in combination with change in length or at constant length, and in virtually any direction relative to the axis of the arm. Both localized and general bending movements were observed. Localized bending typically occurs at the base, while the remainder of the tube foot remains essentially straight and thus pivots about the base. General bending occurs along the entire length of a tube foot, sometimes causing the tip of the tube foot to be oriented at 90° to the base.

During locomotion, the tube feet undergo repeated, stereotyped stepping cycles. An individual tube foot first elongates and bends at the base so that the tip of the foot points in the direction of motion. Once the tube foot is fully protracted, it bends downward until the tip contacts and attaches to the substratum. The tube foot then bends at its base and moves the animal over the point of attachment. In the final phase of the cycle, the tube foot releases its attachment and retracts. The cycle is then repeated. The tube feet do not move synchronously but are coordinated so that those on different arms create movement in a single direction. *L. clathrata* may reach speeds of 2 cm/s when moving across the surface of a crushed oyster shell substratum.

Tube foot movements during burrowing involve bending away from the ambulacrum toward the sides of the

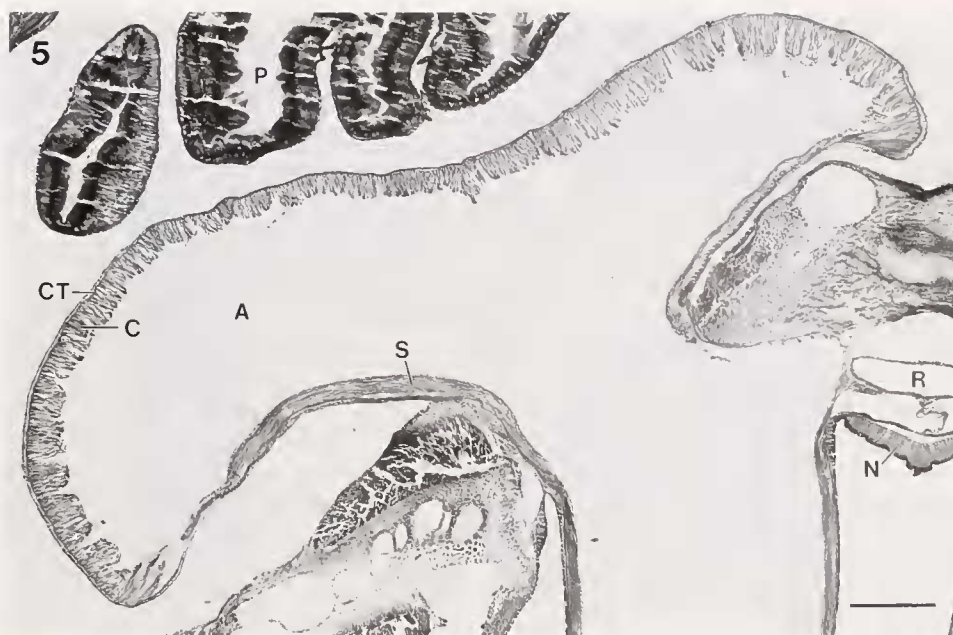


Figure 5. Photomicrograph of a transverse section of an arm from *Luidia clathrata* showing the bilobed ampulla. A thin connective tissue layer (CT) surrounds the circumferential muscles (C) of the ampulla (A). The ampullar seam (S) is visible. Also included are the radial canal (R), radial nerve (N), and a portion of a pyloric cecum (P). Scale bar, 0.25 mm. A 10- μ m-thick paraffin section stained with picro-ponceau and iron hematoxylin was photographed under brightfield illumination.

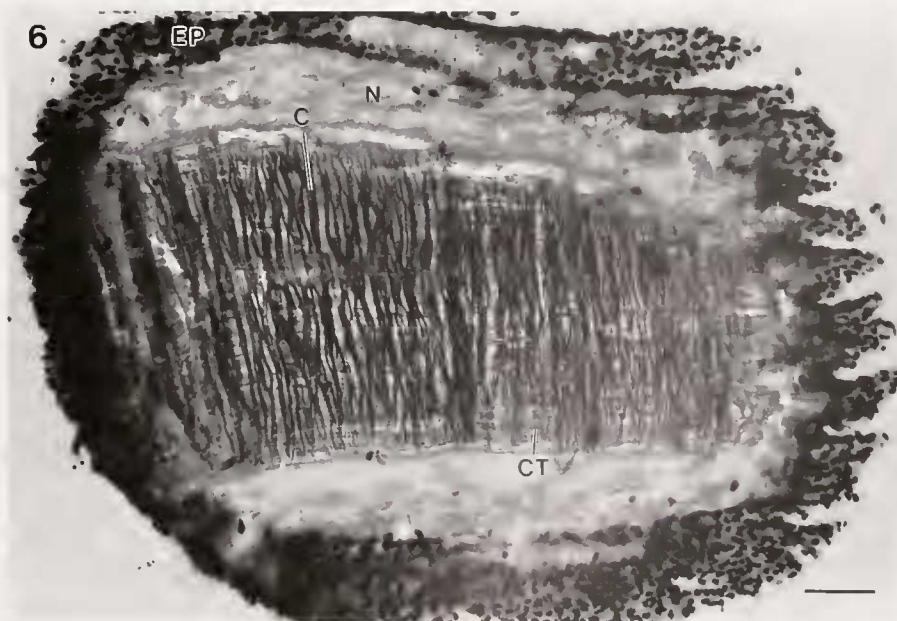


Figure 6. Photomicrograph, taken under polarized light, of a grazing section of an ampulla from *Luidia clathrata*. The long axis of the ampulla is oriented horizontally. The tenuous fibers in the connective tissue layer (CT) are also horizontally disposed, *i.e.*, parallel to the long axis of the ampulla. The robust, vertically oriented circumferential muscle fibers (C) lie underneath the connective tissue layer. The epithelium (EP) and ectoneural nervous tissue layer (N) are also visible. Scale bar, 25 μ m. A 10- μ m-thick paraffin section stained with picro-ponceau and iron hematoxylin was photographed under polarized light.

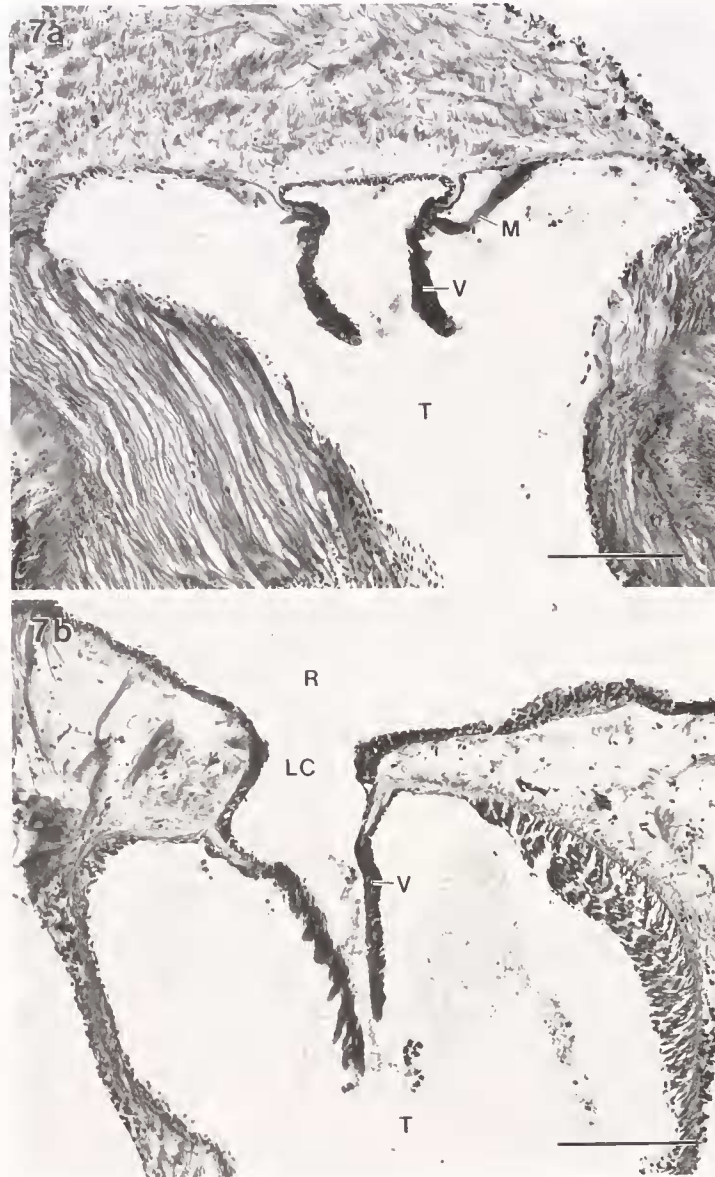


Figure 7. (Top) Photomicrograph of a parasagittal section (vertical section parallel to long axis of the arm) of the arm of *Luidia clathrata* showing the valve at the entrance to the lateral canal. The valve flaps (V) project down from the roof of the tube foot into the tube foot lumen (T). Muscle fibers (M) originate on the roof of the tube foot and insert on the valve flaps. Scale bar, 100 μm . A 10- μm -thick paraffin section stained with picro-ponceau and iron hematoxylin was photographed under brightfield illumination. (Bottom) Photomicrograph of a frontal section of the arm of *Luidia clathrata* showing details of the valve structure. The lateral canal (LC) extends from the radial canal (R) which is oriented horizontally in the figure. The valve flaps (V) project into the tube foot lumen (T) on either side of the entrance of the lateral canal. Scale bar, 100 μm . A 10- μm -thick paraffin section stained with picro-ponceau and iron hematoxylin was photographed under brightfield illumination.

arm. As in locomotion, the burrowing movements of the tube feet are stereotyped and cyclical. An individual tube foot is first protracted downward from the ambulacrum into the sediment. Next, the tube foot bends laterally, sweeping sediment from under the arm. The bending during this phase occurs both at the base of the tube foot

and along its entire length. The tube foot then retracts and bends back toward the ambulacrum to repeat the cycle.

The tube feet are also involved in conveying food down the length of the ambulacral groove to the mouth. As a particle of food approaches a given tube foot, it bends

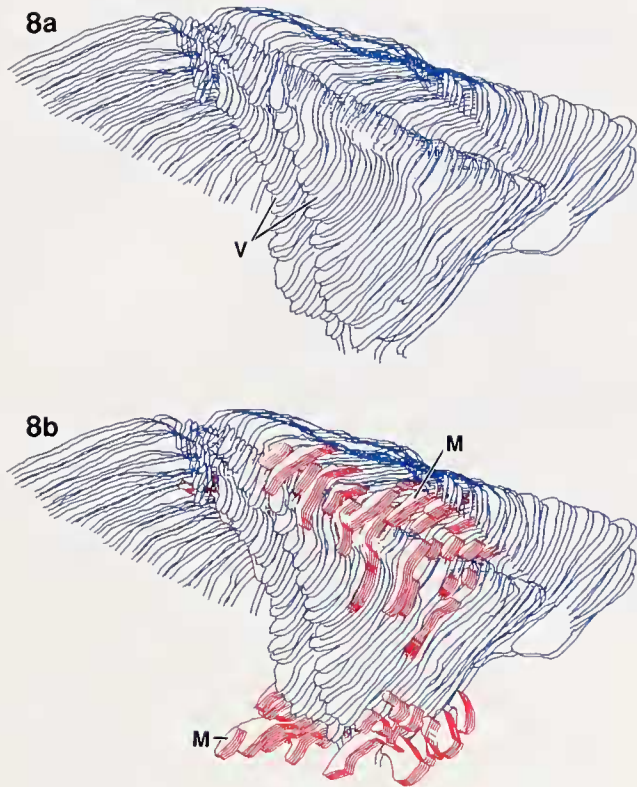


Figure 8. Computer-assisted three-dimensional reconstructions showing the morphology of the valve and valve muscles at the entrance to the lateral canal. Scale bar, 100 μm . (a) Reconstruction showing valve flaps (V) projecting down into the tube foot lumen from the tube foot roof. The flaps are longer adjacent to the wall of the tube foot (not shown, right side of figure) and become shorter as they extend away from the wall along the roof. The opening to the lateral canal is located between the flaps and is thus hidden from view. (b) Reconstruction showing locations of valve muscles (M). Muscles originate on the tube foot roof and insert on the sides of the valve flaps. Additional muscles originate on the tube foot wall and insert near the free edges of the valve flaps.

toward the particle and protracts until the tip adheres to the food. Once attached, the tube foot retracts, pulling the food toward the mouth. At the same time, neighboring tube feet bend toward the food, adhere, and contract. The particle may move beyond the location of a given tube foot while it is still attached, and thus the tube foot elongates further. Whether this elongation involves active protraction by the individual tube foot or passive elongation due to the contraction of neighboring tube feet attached to the same particle remains unclear. The final steps in the cycle are release of the attachment of the tube foot to the particle, and retraction.

The experiments in which the distal portion of the arm and part of the dorsal body wall of *L. clathrata* were removed show that contraction of an ampulla occurs during protraction of its associated tube foot and *vice versa*. Elongation of the tube foot appears to occur only when

its associated ampulla decreases in volume, and expansion of an ampulla occurs only when its associated tube foot contracts. There was no evidence of elongation of the tube feet without contraction of their associated ampullae.

Discussion

Basic tube foot mechanics

The functioning of the tube foot–ampulla complex of *L. clathrata* and *A. articulatus* relies on a hydraulic mechanism similar to that proposed originally by Smith (1945, 1946, 1950a). As described by Kier (1988), in hydraulic systems, force transmission for movement and muscular antagonism results from localized muscle contraction that displaces fluid from one portion of the system to another. The functioning of this system relies on the incompressibility of the hydraulic fluid—in this case the water vascular fluid. Because the fluid is an aqueous liquid and no gas-filled spaces are present, a decrease in volume in one portion of the system results in an increase in volume in another rather than in compression of the fluid.

Tube foot elongation. Elongation of a tube foot is caused by a decrease in volume of its associated ampulla. The musculature of the bilobed ampulla is arranged circumferentially in the ampulla wall. Upon contraction, the muscle decreases the volume of the lumen, displacing water vascular fluid from the lumen into the tube foot. The connective tissue of the ampullar wall plays a crucial role in controlling the shape change of the ampulla (see below). Contraction of the ampullar musculature creates a pressure difference between the lumen of the tube foot and the lumen of the lateral canal. Because the flaps of the valve at the entrance of the lateral canal protrude into the tube foot, the difference in pressure closes the valve, preventing water vascular fluid in the tube foot from backing into the lateral and radial canals. The tube foot is therefore inflated by the fluid displaced from the ampulla, and this increase in volume causes elongation of the tube foot. As in the ampulla, the connective tissue fibers of the tube foot wall play a crucial role in controlling shape change during tube foot elongation (see below). When the tube foot elongates, its longitudinal muscle is extended.

Tube foot shortening. This is caused by contraction of the longitudinal muscle of the tube foot wall. When the tube foot shortens, its volume decreases, and water vascular fluid moves into the ampulla, which expands. Again, the valve at the entrance to the lateral canal closes, preventing fluid from leaking into the water vascular canals, as described above. The increase in fluid volume that expands the ampulla also reextends the circumferential muscle of the ampulla. Thus the circumferential muscle of the ampulla and the longitudinal muscle of the tube foot function as antagonists during length change in the tube foot.

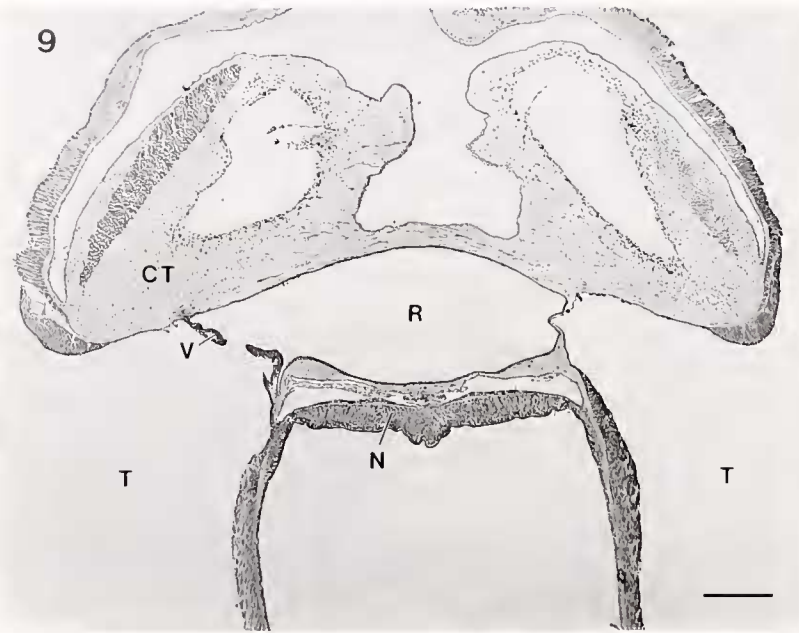


Figure 9. Photomicrograph of a transverse section of the radial canal (R) of *Ludia clathrata*. The connective tissue (CT) of the ambulacrum surrounds the radial canal. No muscle fibers encircle the radial canal. The section is slightly oblique and includes a portion of the valve (V) on the left side of the figure. The radial nerve (N) is located in the center, between the two tube feet (T). Scale bar, 100 μm . A 1- μm -thick glycol methacrylate section stained with Lee's methylene blue–basic fuchsin and photographed under brightfield illumination.

Tube foot bending. Bending of the tube foot involves the same musculature as that used for elongation and shortening. During bending, however, the longitudinal muscles of the tube feet and the circumferential muscles of the ampullae operate synergistically rather than antagonistically. Bending of a hydrostatic structure requires unilateral longitudinal muscle contraction on the inside radius of the bend. The force of this contraction not only bends the structure, but also tends to decrease its length. For bending without shortening, the longitudinal compressional force must be opposed by a resistance to expansion of the ampulla, which prevents displacement of the fluid from the tube foot. Expansion of the ampulla is resisted by contractile activity of the circumferential muscles. Thus, bending requires simultaneous unilateral longitudinal muscle contraction of the tube foot and circumferential muscle contraction of the ampulla. Generation of a localized rather than a more generalized bend is dependent on the degree to which the pattern of activity of the longitudinal musculature of the tube foot is localized. No morphological subdivision of the longitudinal musculature at the base of the tube foot was observed in the species examined in this study.

The role of the radial canal. There is no evidence in the species studied here that the radial canal plays a role in protraction, as has been suggested previously (e.g., Nichols, 1969, 1972). Nichols (1972) described the radial

canal as being highly muscular in modern starfish (species not identified) and capable of receiving fluid from retracted tube feet, and then contracting to distribute the fluid. In the species analyzed in the present study, the radial canal completely lacks musculature. There is no evidence for sphincter muscles or other structures that might allow the radial canal to be partitioned along its length. Further, it is wrapped by the connective tissue and calcite ossicles of the ambulacrum and is thus prevented from expanding. Our results are in agreement with those of Smith (1946) who suggested, on the basis of both direct observation of the tube feet and ampullae and calculations of volume accommodated by the ampullae during tube foot retraction (*Astropecten irregularis*, *Asterias rubens*), that "little, if any fluid enters or leaves the tube foot ampulla system during these movements" (p. 280). The species examined by Nichols (1972) were not identified, so we can say only that participation by the radial canal in tube foot elongation is not a universal feature of the water vascular system of asteroids.

The role of the connective tissue. The connective tissue fibers observed in both the tube foot and the ampullar wall play a crucial role in the mechanics of the tube foot and the ampulla. Consider first the requirement that an increase in pressure (due to contraction of the ampulla) causes elongation of a tube foot. The stress distribution in a pressurized cylinder is such that the hoop stress (which

tends to increase diameter) is twice as large as the longitudinal stress (which tends to increase length) (see Wainwright *et al.*, 1976; Wainwright, 1982; for details). Thus, in a pressurized cylinder lacking fiber reinforcement, the result of increasing pressure is swelling of the diameter of the cylinder, rather than elongation. The crossed-fiber helical array of connective tissue fibers described here reinforces the wall so that an increase in pressure causes an increase in length, rather than diameter. Previous reports (Smith, 1946, 1947) of circular "rings" of connective tissue fibers in the tube foot wall (*Astropecten irregularis*, *Asterias rubens*) are probably the result of misinterpretations of folding of the connective tissue in the wall of retracted tube feet. No circular rings of connective tissue were seen in any of the material from the species examined in this study.

To understand how fiber reinforcement can control shape change, consider a simple geometrical model derived from those described by Chapman (1958), Clark and Cowey (1958), Cowey (1952), and Woodley (1980). The tube foot can be modeled as a right circular cylinder wrapped by a single turn of an inextensible helical fiber. Because the tube foot is essentially cylindrical and the connective tissue fibers are likely to be collagenous and thus quite stiff in tension, the assumptions of this simple model are reasonable. For a helical fiber of given length, the volume of such a cylinder is a function of the length of the cylinder and the fiber angle (the angle that the fiber makes with the long axis of the cylinder) (Fig. 10). The fiber angle of the helical fiber ranges from 90° (when the cylinder length is at a minimum) to 0° (when the cylinder length is at a maximum). The maximum volume occurs at an intermediate length, when the fiber angle is $54^\circ 44'$. If the fiber angle of the cylinder is greater than $54^\circ 44'$, an increase in its volume causes an elongation and a decrease in the fiber angle (see Fig. 10). If the fiber angle is initially less than $54^\circ 44'$, an increase in volume causes the cylinder to shorten. At an angle of $54^\circ 44'$, the stiffness in the hoop direction is double the stiffness in the longitudinal direction, and the fibers therefore resist both an increase in length *and* an increase in diameter. The fiber angle of the crossed-fiber helical array thus determines the shape change that results from an increase in volume of the tube foot.

This model predicts that the fiber angle of the connective tissue fibers in the wall of the tube foot must be greater than $54^\circ 44'$ if the increase in volume due to contraction of the ampulla is to cause elongation. Indeed, a fiber angle of about 67° was measured in the connective tissue fibers of elongated tube feet of *L. clathrata*. Although the fiber angle in retracted tube feet could not be measured or calculated due to the folding of the wall, shortening of the tube foot from the elongated state must increase the fiber angle to greater than 67° .

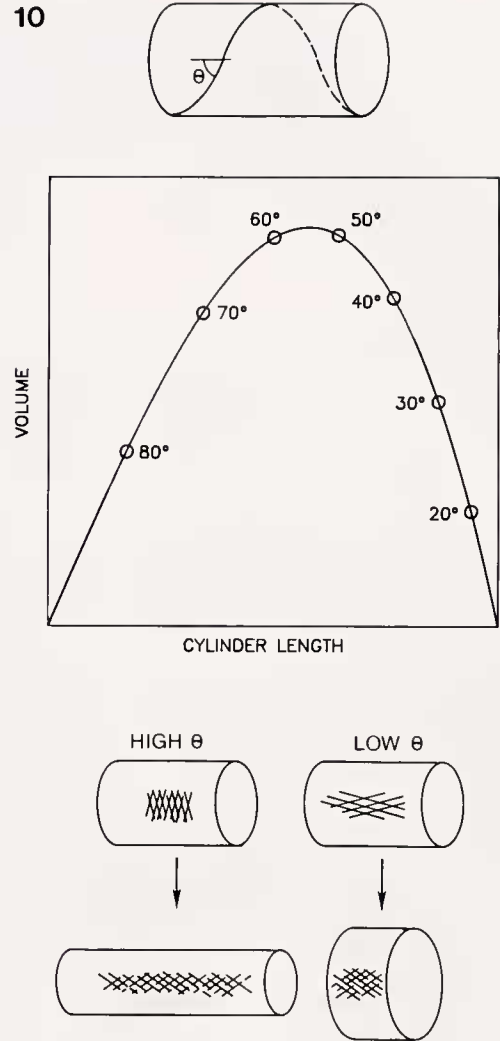


Figure 10. Plot of the length versus volume of a cylinder wrapped by a single inextensible helical fiber. The fiber angle θ , defined as the angle that the helical fiber makes with the long axis of the cylinder, is diagrammed above the plot and is included parametrically on the plot. The volume is maximal when the fiber angle is $54^\circ 44'$. Note that increasing the volume of such a cylinder causes an increase in length only when the initial fiber angle is greater than $54^\circ 44'$ (high θ in diagram below the plot). If the initial fiber angle is less than $54^\circ 44'$, increasing the volume decreases the cylinder length (low θ in diagram below the plot).

The connective tissue fiber reinforcement of the ampullar wall is also functionally important. Contraction of the circumferential musculature of an ampulla increases the pressure of the fluid in the ampulla, generating a stress that tends to elongate the ampulla. Lacking longitudinal muscle fibers (*i.e.*, fibers aligned parallel to the long axis of the ampulla), some other component of the structure must resist this longitudinal stress. The connective tissue fibers of the ampulla are ideally oriented—*i.e.*, parallel to the long axis—and they therefore will resist the longitu-

dinal stress directly. This is a novel example of fiber reinforcement of hydrostatic skeletons; in all other cases, the fibers are in a crossed-fiber helical array (Wainwright *et al.*, 1976; Wainwright, 1982), which allows length change, smooth bending without kinking, and resistance to torsion about the long axis. These are important characteristics of tube feet, but not ampullae, where the most important role of the connective tissue is to resist elongation.

The role of the valve. The valves located at the opening of the lateral canals into the tube feet also have an important function in the water vascular system of asteroids. As described above, the valve flaps are arranged so that an increase in pressure in the tube foot–ampulla complex tends to close the valve, preventing loss of fluid from the tube foot (Märkel and Röser, 1992). Since forceful movement of the tube feet requires significant pressure, fluid is probably lost from the system due to ultrafiltration through the tube foot and ampulla wall (Binyon, 1964, 1984; Prusch and Whorisky, 1976). As a result of active pumping of K^+ , the fluid in the water vascular system is hyperosmotic to the surrounding seawater and to the perivisceral (coelomic) fluid (Binyon, 1962, 1964; Prusch and Whorisky, 1976; Prusch, 1977; Ferguson, 1990a). Because the hydrostatic pressure in temporarily inactive tube feet is low, uptake of water probably occurs across the tube foot wall and ampullar wall. Perhaps uptake in one portion of the water vascular system can replenish the fluid lost in others; the valves could open, allowing fluid to flow via the radial canals. Active opening of the valves is likely because, as described above, the flaps are equipped with muscle fibers with appropriate trajectories. In addition, the madreporite may contribute water to the water vascular system and perivisceral coelom (Ferguson, 1989, 1990b). This mechanism would also require that the valves open to allow fluid to flow from the radial canals into the tube foot–ampulla complex. Active opening of the valves by contraction of the valve musculature, allowing water vascular fluid to flow from contracting tube feet into the radial canal, has been described for ophiuroids (Buchanan and Woodley, 1963; Woodley, 1980), asteroids (Nichols, 1972), and for echinoids under extreme conditions (Märkel and Röser, 1992). For the species studied here, our observations (described earlier) suggest that the tube foot–ampulla complex functions as an autonomous unit during normal activity. Significant flow of water vascular fluid in and out of the radial canal during normal movement appears unlikely.

Implications for neural control. The anatomy, function, and coordination of the nervous system of the tube foot–ampulla complex of asteroids has received considerable attention previously (Smith, 1945, 1946, 1950a, 1950b; Cobb, 1967, 1970, 1987; Cavey and Wood, 1981). Several points arising from the analysis of the present study have

not been previously considered in discussions of the neural control of the tube foot–ampulla complex. Although bending of the tube foot in any direction was known to require localized control of the musculature of the tube foot, the role of synergistic ampulla muscle contraction in providing the support required for bending was not recognized. These two muscle groups operate sequentially during elongation and retraction, and simultaneously during bending movements, so their neural control is complex.

In addition, complexity of nervous control is also implied by the ability of the tube feet either to bend locally and pivot around the base or to form a more generalized bend along the length of the tube foot. In a study of the innervation of the tube feet and ampullae of *Astropecten irregularis*, Smith (1950b) observed a separate innervation restricted to the muscle at the base of the tube foot (which he referred to as postural muscles), as well as a distinct innervation of the musculature of the tube foot wall (which he called retractor muscles). This pattern of innervation is to be expected if the musculature at the base of the tube foot acts alone to create a localized bending while that of the tube foot wall is responsible for more generalized bending.

Acknowledgments

The authors are grateful to S. Johnsen and K. K. Smith for comments on the manuscript. This research was supported by a National Science Foundation Presidential Young Investigator Award (DCB-8658069) and NSF Research Experiences for Undergraduates Supplement.

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